Research Article

Synthesis and Antimicrobial and Antioxidant Activities of Some New 5-(2-Methyl-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-amine Derivatives

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A series of 5-(2-methyl-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-amine derivatives (**3–5**) were synthesized. These previously unknown compounds were characterized by spectral studies and elemental analysis. These compounds were evaluated for their antimicrobial and antioxidant activities. Among all the compounds tested **5d** exhibited promising antibacterial, antifungal, radical scavenging, and ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) activities, whereas the compounds **3b**, **4c**, and **5e** exhibited good FRAP and metal chelating activities. In general compounds containing chloro and methyl substituent exhibited better antimicrobial and antioxidant activities.

1. Introduction

Indole and its analogs display remarkable pharmacological activities like Hepatitis C Virus NS5B protein inhibitiors [1–3] peroxisome proliferator-activated receptor γ (PPAR-γ) agonist [4], antiproliferative against human tumor cells [5, 6], antibacterial, antifungal [7, 8], and anticoccidal [9] activities. On the other hand, oxadiazoles possess desirable electronic and charge transport properties into which various functional groups can be easily introduced. Considerable evidence has been accumulated during the last decades demonstrating the various pharmacological properties of its isomer 1,3,4-oxadiazole which includes anticonvulsant [10], tuberculostic [11], analgesic and ulcerogenicity [12], antibacterial, antifungal, diuretic [13], antimiotic [14], anti-inflammatory [15], anti-HIV [16], nonpeptide angiotensin II receptor antagonists [17], tyrosinase inhibition [18], nematocidal, insecticidal, and herbicidal [19] activities. All of which can be attributed to its characteristic properties of electrophilic substitution, and nucleophilic substitution, thermal and photochemical reactions due to the presence of -N=C-O- group. The importance of substituted indolyloxadiazoles is well known for various applications. Indolyl-1,3,4-oxadiazoles, triazoles, and pyrazoles have been reported as antimicrobials [20, 21].

Indole substituted with oxadiazoles or triazoles at positions 1 and/or 3 were found to exhibit enhanced biological activities [22]. In the perspective of bioactivities of the abovementioned structures and in search of novel antimicrobials and antioxidants, we herein report synthesis of novel bis (indolyl) analogues.

2. Results and Discussion

The synthetic strategy was planned as depicted in Scheme 1. The requisite starting materials 5-(2-methyl-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-amine (**1**) were prepared by the cyclocondensation of 2-methyl-1*H*-indol-3-carbohydrazide with cyanogen bromide in ethanol under reflux conditions [23] and 5-substituted 2-phenyl-1*H*-indol-3-carboxaldehydes **2a–c** were prepared by the previously reported method [24]. Condensation of compound **1** with **2a–c** in methanol, under reflux temperature afforded the key intermediate N-[(5-substituted 2-phenyl-1*H*-indol-3-yl) methylene]-5-(2-methyl-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-amines (**3a–c**).

Compounds **3a–c** on cyclocondensation with easily available and economically viable reagents such as thioglycolic acid, phenyl acetyl chloride, and chloro acetyl chloride
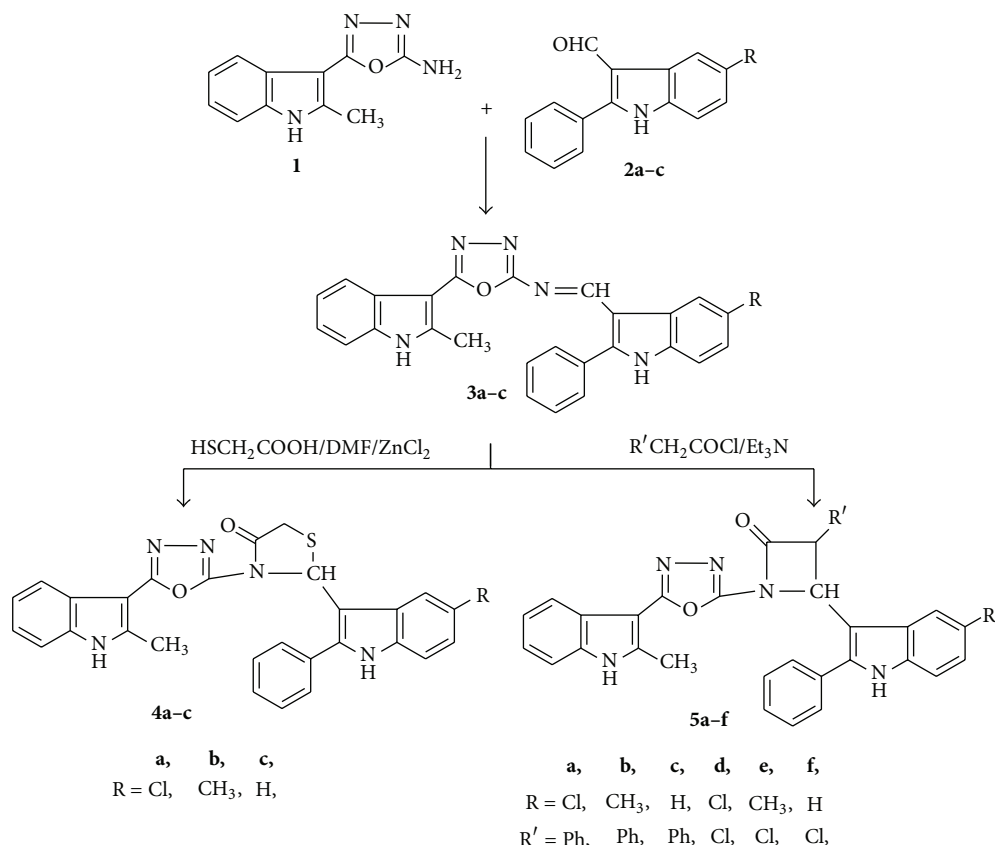
SCHEME 1: Schematic pathway for the synthesis of title compounds.

yielded 2-(5-substituted 2-phenyl-1*H*-indol-3-yl)-3-[5-(2-methyl-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-yl] thiazolidin-4-ones (**4a–c**), 4-(5-substituted 2-phenyl-1*H*-indol-3-yl)-1-[5-(2-methyl-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-yl]-3-phenylazetidin-2-ones (**5a–c**), and 3-chloro-4-(5-substituted 2-phenyl-1*H*-indol-3-yl)-1-[5-(2-methyl-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-yl]azetidin-2-ones (**5d–f**), respectively.

2.1. Antimicrobial Activities.

Antimicrobial screening of the compounds (**1** and **3–5**) was performed by cup-plate method at a concentration of 1 mg/mL following reported procedure [25]. The bacteria *Escherichia coli*, *Bacillus subtilis*, and *Klebsiella pneumonia* and fungal *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigates* were used. The zones of inhibition were compared with the standards; streptomycin and fluconazole were used as standards for antibacterial and antifungal activities, respectively. The results are presented in Table 1.

The structure-activity relationship (SAR) revealed that the compounds with chlorine substituent at C-5 position of indole and in azetidinone system are important for enhancing the antimicrobial activity, whereas methyl substituent at C-5 position of indole is found to reduce the antimicrobial activity. Thus, compound **5d** has been found to exhibit maximum zone of inhibition against all the bacteria and fungi. Replacement of azetidinone nucleus by thiazolidinone

system leads to formation of compounds **4a–c** with decreased antimicrobial activity. On the other hand, compounds **3a** and **4a** exhibited less zone of inhibition compared to **5d** against all bacterial strains and the fungus *A. niger*. Removal of chlorine substituent at C-5 position of indole (compound **5f**) leads to decrease in antibacterial activity against *B. subtilis*. Replacement of chlorine at C-5 position of indole by methyl group and azetidine system by thiazolidinone ring (compound **4b**) exhibited same zone of inhibition as that of **5d** against bacterial strain *K. pneumonia* and showed slight decrease in antifungal activity against *A. flavus*. Substitution of chlorine by phenyl group in **5d** (compound **5a**) leads to decrease in antifungal activity against *A. flavus* and *A. fumigates*. On the same line, when chlorine at C-5 position of indole in **5d** was replaced by methyl group (compound **5e**) slight decrease in antifungal activity against *A. fumigates* was noticed. However, none of the compounds exhibited better antimicrobial activity compared with standards.

2.2. Antioxidant Activities

2.2.1. 1,1-Diphenyl-2-picryl Hydrazyl (DPPH) Radical Scavenging Activity (RSA).

Investigation of the RSA of the test compounds (**1** and **3–5**) was conducted as described by Hatano's and colleagues [26] and the results were compared with the

TABLE 1: Antimicrobial activities of the compounds (**1** and **3–5**).

Compound no.	R	R′	Diameter of zone of inhibition in mm#					
			Antibacterial activity			Antifungal activity		
			E. coli	B. subtilis	K. pneumonia	A. niger	A. flavus	A. fumigates
1	—	—	14 ± 1.63	12 ± 1.31	14 ± 1.88	17 ± 1.60	14 ± 1.76	11 ± 1.81
3a	Cl	—	10 ± 2.29	14 ± 1.27	06 ± 1.93	09 ± 1.41	12 ± 1.06	13 ± 1.68
3b	CH$_3$	—	05 ± 1.83	09 ± 1.25	13 ± 1.85	08 ± 0.67	11 ± 1.60	18 ± 1.43
3c	H	—	02 ± 1.01	07 ± 1.06	12 ± 1.35	00 ± 0.00	05 ± 1.35	03 ± 1.81
4a	Cl	—	14 ± 2.00	14 ± 1.72	10 ± 2.80	18 ± 1.82	13 ± 1.82	08 ± 2.00
4b	CH$_3$	—	06 ± 1.43	03 ± 1.39	15 ± 2.25	08 ± 1.96	19 ± 2.16	07 ± 1.76
4c	H	—	10 ± 2.05	09 ± 1.02	07 ± 1.54	06 ± 1.49	04 ± 1.47	09 ± 1.76
5a	Cl	Ph	03 ± 1.57	14 ± 1.73	04 ± 1.51	12 ± 1.88	19 ± 0.94	17 ± 1.72
5b	CH$_3$	Ph	13 ± 2.15	10 ± 1.56	05 ± 1.61	05 ± 1.39	01 ± 0.76	13 ± 1.68
5c	H	Ph	07 ± 1.24	11 ± 1.49	12 ± 1.96	9 ± 1.92	14 ± 1.48	12 ± 0.91
5d	Cl	Cl	16 ± 2.23	15 ± 2.16	15 ± 1.96	19 ± 1.72	20 ± 1.68	18 ± 1.49
5e	CH$_3$	Cl	02 ± 1.23	08 ± 1.15	05 ± 1.48	07 ± 1.72	05 ± 1.68	17 ± 1.50
5f	H	Cl	10 ± 1.60	14 ± 1.35	07 ± 1.28	12 ± 0.95	15 ± 1.12	08 ± 1.39
Std-1	—	—	17 ± 1.80	16 ± 1.92	16 ± 2.29	—	—	—
Std-2	—	—	—	—	—	20 ± 1.61	22 ± 1.61	19 ± 1.81

Including diameter of well#, control (DMF) = no activity, Std-1 = streptomycin, Std-2 = fluconazole.

results obtained using standards 2-tert-butyl-4-methoxy phenol (butylated hydroxyl anisole, BHA), 2-(1,1-dimethylethyl)-1,4-benzenediol (tertiary butylated hydroquinone, TBHQ), and ascorbic acid (AA).

From the RSA results of test compounds (Figures 1 and 2) it could be seen that introduction of chlorine substitution at C-5 position of indole ring (compound **3a**) enhances the RSA (72.35% at 100 μg/mL), whereas the introduction of methyl group at same position of indole (compound **3b**) leads to decrease in RSA (55.28% at 100 μg/mL). Replacement of the azomethine group in compounds **3** by thiazolidinone nucleus (compounds **4**) resulted in a decrease in RSA. But replacement of azomethine group by azetidinone ring with the chlorine substitution at C-5 of indole (compound **5d**) was found to enhance the RSA (74.15% at 100 μg/mL). Replacement of chlorine substituent by phenyl group in azetidinone system (compound **5a**) or replacement of chlorine substitution by phenyl group in azetidinone ring and methyl group at C-5 position of indole (compound **5b**) was found to decrease RSA (62.12% at 100 μg/mL).

2.2.2. Ferric Ions (Fe^{3+}) Reducing Antioxidant Power (FRAP).
The ferric ion (Fe^{3+}) is a relatively biologically inactive form of iron. However, it can be reduced to active Fe^{2+}, depending on the condition, particularly pH [27] and oxidized back through Fenton type reaction with production of hydroxyl radical or Haber-Weiss reaction with the generation of superoxide anions. Reducing power is to measure the reductive ability of antioxidant. It is evaluated by the transformation of Fe^{3+} to Fe^{2+} by donation of an electron, in the presence of test compounds. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

Determination of the reducing power of the compounds (**1** and **3–5**) at different concentrations (25, 50, 75, and
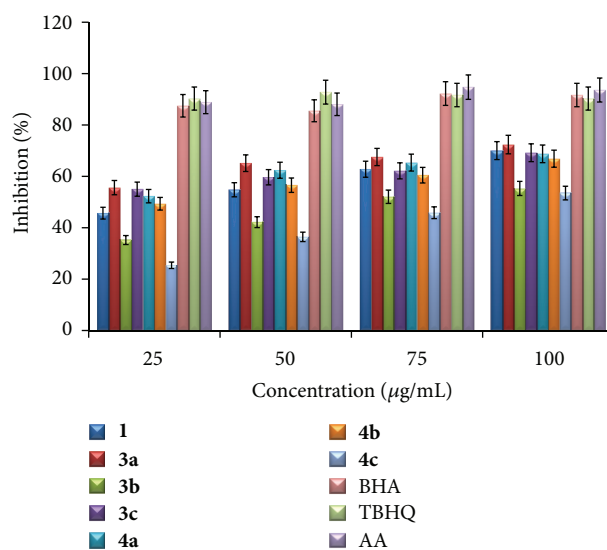

FIGURE 1: RSA of the compounds (**1** and **3-4**).

100 μg/mL) was conducted by Oyaizu method [28] using BHA, TBHQ, and AA as standards. The FRAP results (Figures 3 and 4) revealed that methyl substitution at C-5 position of indole (compound **3b**) leads to increase in FRAP, whereas introduction of chlorine substituent at C-5 of indole (compound **3d**) leads to decrease in FRAP. Replacement of azomethine group by thiazolidinone ring (compound **4a**) increases the FRAP, but introduction of either chlorine or methyl substituent (compound **4a** and **4b**) leads to decrease in FRAP, whereas replacement of azomethine group by azetidinone ring with chloro substitution (compound **5e**) exhibited promising FRAP. Introduction of chlorine or
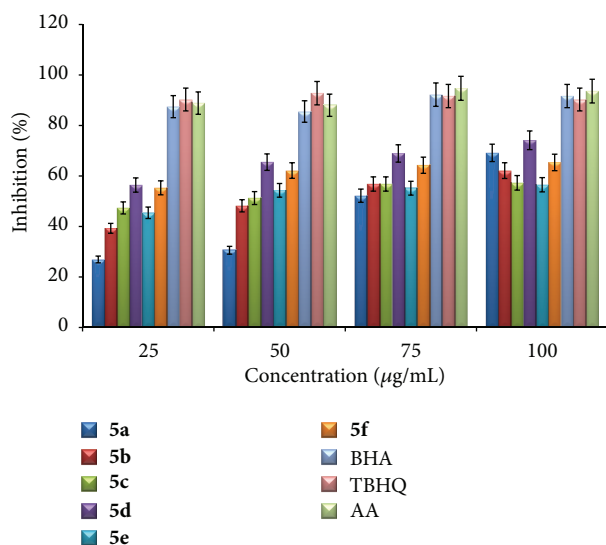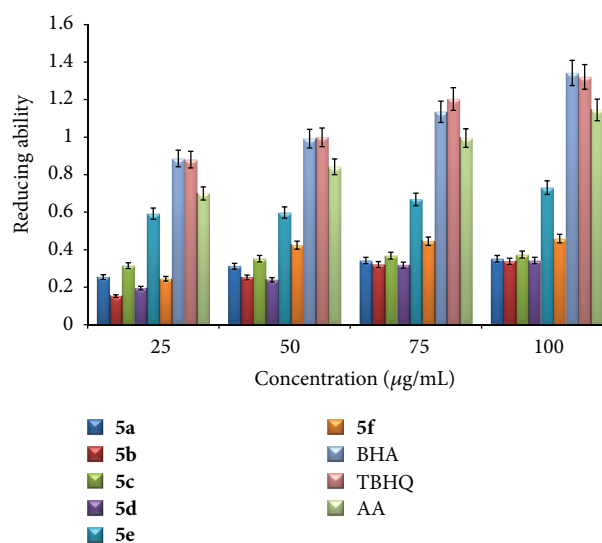
FIGURE 2: RSA of the compounds (5a–f).

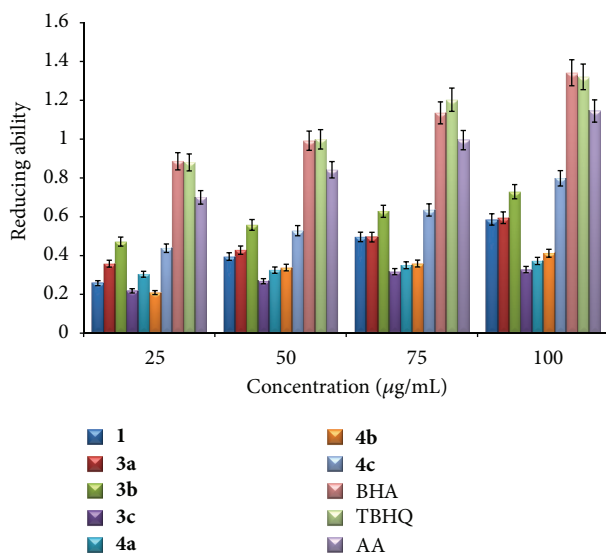FIGURE 4: Ferric (Fe^{3+}) ions reducing capacity of the compounds (5a–f).

FIGURE 3: Ferric (Fe^{3+}) ions reducing capacity of the compounds (1 and 3-4).
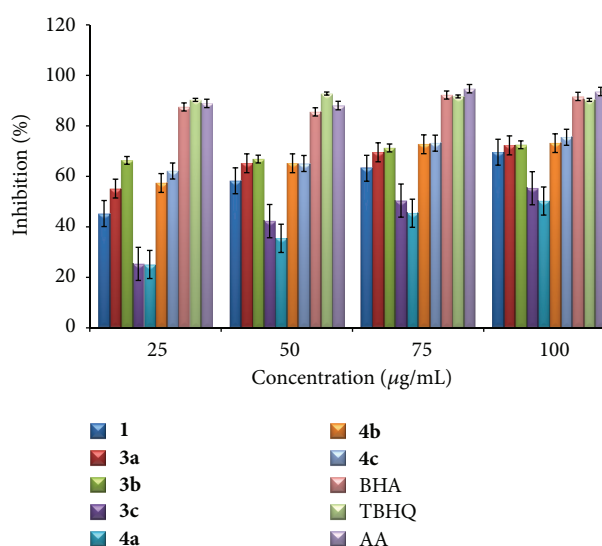

FIGURE 5: Metal chelating activity of the compounds (1 and 3-4).

methyl substituent at C-5 position of indole and replacement of chlorine by phenyl group in azetidinone ring (compounds 5a and 5b) lead to decrease in FRAP.

2.2.3. Ferrous (Fe^{2+}) Metal Ion Chelating Activity. The chelating effect of ferrous ions (Fe^{2+}) towards the test compounds (1 and 3–5) and standards were determined by following Dinis method [29] and the results were compared with standards BHA, TBHQ, and AA. Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted, as a result the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction.

In metal chelating activity (Figures 5 and 6), the SAR study revealed that introduction of chloro or methyl substitution at C-5 position of indole (compounds 3a and 3b) along with azomethine group leads to improved metal chelating activity (71.24 and 72.51% at 100 μg/mL, resp.). Replacement of azomethine group by thiazolidinone ring (compound 4c) showed higher activity (75.48% at 100 μg/mL). Introduction of methyl group (compound 4b) leads to slight decrease in metal chelating activity, whereas introduction of chlorine at C-5 position of indole (compound 4a) drastically reduced the metal chelating activity. Introduction of chlorine substituent at C-5 position of indole (compound 5d) was found to increase metal chelating activity (66.25% at 75 μg/mL), whereas the introduction of either chloro or methyl substituent at C-5 position of indole and phenyl substitution in
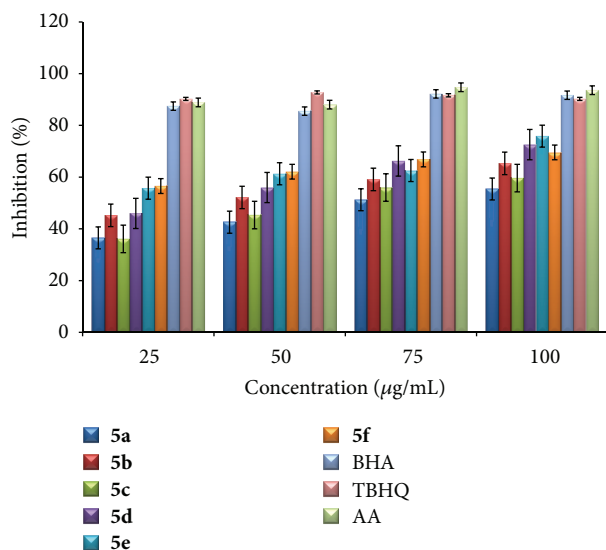
FIGURE 6: Metal chelating activity of compounds (5a–f).

azetidinone ring (compound 5a and 5b) lead to decrease in metal chelating activity. Replacement of azomethine group by azetidine ring with methyl substituent at C-5 position of indole and chloro substitution in azetidinone ring (compound 5e) exhibited higher metal chelating activity (75.84% at 100 μg/mL). However, none of the compounds showed activity better than the standards.

3. Conclusion

We have reported the synthesis and antimicrobial and antioxidant activities of novel bis (indolyl) compounds 1, 3a–c, 4a–c, and 5a–f. Compounds bearing chloro substitution exhibited significant antimicrobial and antioxidant activities. Among the synthesized compounds, 5d was found to be most active against all the microorganism tested and also exhibited promising RSA and FRAP suggesting that the presence of chloro substituent and the azetidinone nucleus are responsible for activities among the synthesized compounds.

4. Experimental Protocol

All the chemicals and reagents were commercially obtained and used by further purification. Melting points were determined by an open capillary method and are uncorrected. Purity of the compounds was checked by TLC using silica gel-G coated aluminium plates (Merck) and spots were visualized by exposing the dry plates in iodine vapours. The IR (KBr) spectra were recorded with a Perkin-Elmer spectrum one FT-IR spectrometer. The ^{1}H NMR (DMSO-d_6) spectra were acquired with a Bruker NMR (500 MHz) and the chemical shifts are expressed in ppm (δ scale) downfield from TMS. Mass spectra were obtained with a JEOL GCMATE II GC-MS mass spectrometer. Elemental analysis was carried out using Flash EA 1112 series elemental analyzer.

4.1. General Procedure for the Synthesis of 5-(2-Methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-amine (1). A mixture of 2-methyl-1*H*-indol-3-carbohydrazide (0.01 mol) and cyanogen bromide (0.01 mol) in EtOH was refluxed with stirring for 90 min. The reaction mixture was then cooled and neutralized by sodium bicarbonate solution. The product separated was filtered, dried and recrystallized in ethanol to afford pure 1. Yield: 75%; mp 285-86°C; R_f 0.65 ethyl acetate : methanol (6 : 4) mixture; FTIR (KBr) cm^{-1}: 3441 (NH), 3278 (NH$_2$), 1602 (C=N), 1074 (C–O–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.01 (s, 1H, indole NH), 7.00–8.05 (m, 4H, Ar-H), 5.85 (s, 2H, NH$_2$), 2.82 (s, 3H, CH$_3$); MS (EI): *m/z* 214 (M$^+$). Anal. % C$_{11}$H$_{10}$N$_4$O: C, 61.67; H, 4.71; N, 26.15. Found: C, 61.64; H, 4.69; N, 26.14.

4.2. General Procedure for the Synthesis of N-[(5-Substituted 2-phenyl-1H-indol-3-yl)methylene]-5-(2-methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-amines (3a–3c). Compound 1 (0.01 mol) and 5-substituted 2-phenyl-1*H*-indol-3-carbaldehydes (2a–2c) (0.01 mol) were refluxed in methanol (25 mL) on water bath for 8 hrs. Excess of solvent was removed under reduced pressure. After cooling the reaction mixture to room temperature light yellowish crystalline product separated out was collected by filtration and recrystallized in alcohol.

N-((5-Chloro-2-phenyl-1H-indol-3-yl)methylene)-5-(2-methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-amine (3a). Yield: 75%, mp 228-29°C; R_f 0.77 ethyl acetate : acetone (6 : 4) mixture; FTIR (KBr) cm^{-1}: 3310 (NH), 3289 (NH), 1626 (C=N), 1096 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.00, 11.75 (s, 2H, indole NH), 8.52 (s, 1H, N=CH), 6.92–7.98 (m, 12H, Ar-H), 2.25 (s, 3H, CH$_3$); ^{13}C-NMR (DMSO-d_6, 125 MHz, δ): 160.3, 158.2, 156.4, 142.5, 134.9, 134.0, 133.0, 132.1, 130.3, 129.3, 128.5, 127.2, 126.8, 126.1, 125.8, 124.3, 123.0, 122.6, 122.1, 121.6, 121.4, 119.8, 119.5, 15.4; MS (EI): *m/z* 451 (M$^+$); 452 (M$^+$+2). Anal. % C$_{26}$H$_{18}$N$_5$OCl: C, 69.10; H, 4.01; N, 15.50. Found: C, 68.64; H, 3.99; N, 15.24.

5-(2-Methyl-1H-indol-3-yl)-N-((5-methyl-2-phenyl-1H-indol-3-yl)methylene)-1,3,4-oxadiazol-2-amine (3b). Yield: 76%, mp 240-41°C; R_f 0.58 ethyl acetate : acetone (6 : 4) mixture; FTIR (KBr) cm^{-1}: 3210 (NH), 3200 (NH), 1620 (C=N), 1085 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 11.93, 11.52 (s, 2H, indole NH), 8.45 (s, 1H, N=CH), 6.98–7.95 (m, 12H, Ar-H), 3.13 (s, 3H, CH$_3$), 2.43 (s, 3H, CH$_3$); Anal. % C$_{27}$H$_{21}$N$_5$O: C, 75.16; H, 4.91; N, 16.23. Found: C, 75.14; H, 4.79; N, 15.65.

5-(2-Methyl-1H-indol-3-yl)-N-[(2-phenyl-1H-indol-3-yl)methylene]-1,3,4-oxadiazol-2-amine (3c). Yield: 78%, mp 212-13°C; R_f 0.68 ethyl acetate : acetone (6 : 4) mixture; FTIR (KBr) cm^{-1}: 3287 (NH), 3215 (NH), 1609 (C=N), 1086 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.10, 11.92 (s, 2H, indole NH), 8.61 (s, 1H, N=CH), 6.90–7.99 (m, 13H, Ar-H), 2.37 (s, 3H, CH$_3$); Anal. % C$_{26}$H$_{19}$N$_5$O: C, 74.80; H, 4.59; N, 16.78. Found C, 74.76; H, 4.62; N, 15.76.

4.3. General Procedure for the Synthesis of 2-(5-Substituted 2-phenyl-1H-indol-3-yl)-3-[5-(2-methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-yl]thiazolidin-4-ones **(4a–4c)**. To the solution of compounds **3a**, **3b,** and **3c** (0.02 mol) in DMF (45 mL), thioglycolic acid (0.02 mol) and anhydrous zinc chloride (0.02 mol) were added and refluxed for 6 hrs. After the completion of reaction, excess of solvent was removed under reduced pressure, cooled to room temperature, and poured onto crushed ice. The solid product thus separated was filtered, washed with cold water, and recrystallized in alcohol to afford (**4a–4c**).

2-(5-Chloro-2-phenyl-1H-indol-3-yl)-3-(5-(2-methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-yl)thiazolidin-4-one **(4a)**. Yield: 72%, mp 272-73°C; R_f 0.57 ethyl acetate : benzene (8 : 2) mixture; FTIR (KBr) cm^{-1}: 3344 (NH), 3302 (NH), 1715 (C=O), 1627 (C=N), 1097 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.12, 11.79 (s, 2H, indole NH), 8.72 (s, 1H, CHCO), 6.91–8.05 (m, 12H, Ar-H), 4.81 (s, 2H, N-CH), 2.43 (s, 3H, CH$_3$); ^{13}C-NMR (DMSO-d_6, 125 MHz, δ): 168.5, 159.2, 154.5, 143.9, 135.0, 134.1, 133.0, 132.8, 131.1, 130.1, 127.8, 127.6,126.8, 126.3, 125.4, 125.1, 123.0, 122.8, 122.0, 121.8, 120.0, 119.0, 55.9, 35.2, 15.5; MS (EI): *m/z* 525 (M$^+$); 527 (M$^+$ +2). C$_{28}$H$_{20}$N$_5$O$_2$SCl: C, 63.93; H, 3.83; N, 13.31. Found: C, 63.90; H, 3.79; N, 13.30.

3-[5-(2-Methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-yl]-2-(5-methyl-2-phenyl-1H-indol-3-yl)thiazolidin-4-one **(4b)**. Yield: 67%, mp 243-44°C; R_f 0.53 ethyl acetate : ethanol (8 : 2) mixture; FTIR (KBr) cm^{-1}: 3230 (NH), 3205 (NH), 1743 (C=O), 1637 (C=N), 1086 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.03, 11.67 (s, 2H, indole NH), 8.59 (s, 1H, CHCO), 6.96–8.25 (m, 12H, Ar-H), 4.76 (s, 2H, N-CH), 3.05 (s, 3H, CH$_3$), 2.40 (s, 3H, CH$_3$); Anal. % C$_{29}$H$_{23}$N$_5$O$_2$S: C, 68.89; H, 4.59; N, 13.85. Found: C, 68.90; H, 4.60; N, 13.79.

3-[5-(2-Methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-yl]-2-(2-phenyl-1H-indol-3-yl)thiazolidin-4-one **(4c)**. Yield: 68%, mp 250-51°C; R_f 0.68 ethyl acetate : ethanol (8 : 2) mixture; FTIR (KBr) cm^{-1}: 3330, 3298 (N-H); 1715 (C=O); 1627 (C=N); 1097 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.00, 11.69 (s, 2H, indole NH); 8.68 (s, 1H, CHCO), 6.81–8.00 (m, 13H, Ar-H); 4.31 (s, 2H, N-CH); 2.62 (s, 3H, CH$_3$); Anal. % C$_{28}$H$_{21}$N$_5$O$_2$S: C, 68.41; H, 4.31; N, 14.25. Found: C, 68.35; H, 4.31; N, 14.24.

4.4. General Procedure for the Synthesis of 4-(5-Substituted 2-phenyl-1H-indol-3-yl)-1-[5-(2-methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-yl]-3-phenylazetidin-2-ones **(5a–5c)**. To Schiff's base (**3a–3c**) (0.02 mol) in dry benzene (30 mL) containing few drops of triethyl amine, phenyl acetyl chloride (0.02 mol) was added with stirring during 10 mins at room temperature. After the addition was over, reaction mixture was refluxed for 1 hr. Triethyl amine hydrochloride formed was filtered off and washed several times with dry benzene. The filtrate and washings were combined and concentrated under reduced pressure. After cooling at room temperature the product

obtained was filtered, washed with petroleum ether (40 : 60) and recrystallized in aqueous ethanol.

4-(5-Chloro-2-phenyl-1H-indol-3-yl)-1-[5-(2-methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-yl)-3-phenylazetidin-2-one **(5a)**. Yield: 71%, mp 158-59°C; R_f 0.59 ethyl acetate : acetone (6 : 4) mixture; FTIR (KBr) cm^{-1}: 3430 (NH), 3387 (NH), 1765 (C=O), 1626 (C=N), 1084 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 11.93, 11.58 (s, 2H, indole NH), 8.43 (d, 1H, CH-N), 7.00–8.06 (m, 17H, Ar-H), 5.58 (d, 1H, CH-Ph), 2.21 (s, 3H, CH$_3$): MS (EI): *m/z* 569 (M$^+$); 571 (M$^+$ +2). Anal. % C$_{34}$H$_{24}$N$_5$O$_2$Cl: C, 71.64; H, 4.24; N, 12.29. Found: C, 71.60; H, 4.21; N, 12.16.

1-[5-(2-Methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-(5-methyl-2-phenyl-1H-indol-3-yl]-3-phenylazetidin-2-one **(5b)**. Yield: 64%, mp 155-56°C; R_f 0.67 ethyl acetate : acetone (6 : 4) mixture; FTIR (KBr) cm^{-1}: 3341 (NH), 3307 (NH), 1743 (C=O), 1620 (C=N), 1093 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.05, 11.85 (s, 2H, indole NH), 8.23 (d, 1H, CH-N), 7.09–8.00 (m, 17H, Ar-H), 4.57 (d, 1H, CH-Ph), 2.65 (s, 3H, CH$_3$), 2.43 (s, 3H, CH$_3$); Anal. % C$_{35}$H$_{27}$N$_5$O$_2$: C, 76.48; H, 4.95; N, 12.74. Found: C, 76.37; H, 4.83; N, 12.66.

1-[5-(2-Methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-yl)-3-phenyl-4-(2-phenyl-1H-indol-3-yl)azetidin-2-one **(5c)**. Yield: 70%, mp 159-60°C; R_f 0.76 ethyl acetate: acetone (6 : 4) mixture; FTIR (KBr) cm^{-1}: 3200 (NH), 3158 (NH), 1712 (C=O), 1616 (C=N), 1093 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 11.99, 11.56 (s, 2H, indole NH), 8.13 (d, 1H, CH-N), 6.92–7.96 (m, 17H, Ar-H), 5.00 (d, 1H, CH-Ph), 2.31 (s, 3H, CH$_3$); Anal. % C$_{33}$H$_{24}$N$_5$O$_2$: C, 75.85; H, 4.63; N, 13.40. Found: C, 75.74; H, 4.57; N, 13.48.

4.5. General Procedure for the Synthesis of 3-Chloro-4-(5-substituted 2-phenyl-1H-indol-3-yl)-1-[5-(2-methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-yl]azetidin-2-ones **(5d–5f)**. To the solution of compounds (**3a–3c**) (0.02 mol) in dioxane, chloroacetyl chloride (0.04 mol) and triethyl amine (0.04 mol) were added with constant stirring at 0–5°C temperature during 10 mins. After the addition was over, the reaction mixtures were refluxed for 8–10 hrs and the excess of solvent was removed under reduced pressure. After cooling at room temperature the product obtained was filtered, washed with dioxane, dried, and recrystallized in alcohol to get pure (**5d–5f**).

3-Chloro-4-(5-chloro-2-phenyl-1H-indol-3-yl)-1-(5-(2-methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-yl)azetidin-2-one **(5d)**. Yield: 74%, mp 273-74°C; R_f 0.71 ethyl acetate : benzene (6 : 4) mixture; FTIR (KBr) cm^{-1}: 3411(NH), 3357 (NH), 1716 (C=O), 1628 (C=N), 1073 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.02, 11.89 (s, 2H, indole NH), 8.75 (d, 1H, CHCO), 7.07–8.06 (m, 12H, Ar-H), 5.31 (d, 1H, N-CH), 2.23 (s, 3H, CH$_3$); ^{13}C-NMR (DMSO-d_6, 125 MHz, δ): 167.2, 159.3, 156.4,

144.6, 134.8, 133.9, 133.2, 131.5, 131.3, 130.2, 129.9, 129.8, 129.4, 128.1, 127.5, 127.0, 126.8, 125.9, 125.1, 123.9, 123.4, 122.3, 122.0, 121.5, 120.1, 120.0 64.5, 63.1, 15.9; MS (EI): m/z 527 (M^+); 529 (M^+ +2): 531 (M^+ +4). Anal. % $C_{28}H_{19}N_5O_2Cl_2$: C, 63.65; H, 3.62; N, 13.25. Found: C, 63.68; H, 3.55; N, 13.12.

3-Chloro-1-(5-(2-methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-yl)-4-(5-methyl-2-phenyl-1H-indol-3-yl)azetidin-2-one **(5e)**. Yield: 69%, mp 255-56°C; R_f 0.58 ethyl acetate : benzene (6 : 4) mixture; FTIR (KBr) cm^{-1}: 3371, 3312 (N-H); 1719 (C=O); 1620 (C=N); 1087 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.95, 11.64 (s, 2H, indole NH); 8.67 (d, 1H, CHCO); 6.97–8.16 (m, 12H, Ar-H); 4.34 (d, 1H, N-CH); 2.43 (s, 3H, CH$_3$); 2.13 (s, 3H, CH$_3$); Anal. %: $C_{29}H_{22}N_5O_2Cl$: C, 68.57; H, 4.37; N, 13.79. Found: C, 68.50; H, 4.29; N, 13.84.

3-Chloro-1-[5-(2-methyl-1H-indol-3-yl)-1,3,4-oxadiazol-2-yl]-4-(2-phenyl-1H-indol-3-yl)azetidin-2-one **(5f)**. Yield: 72%, mp 233-34°C; R_f 0.66 ethyl acetate : benzene (6 : 4) mixture; FTIR (KBr) cm^{-1}: 3278 (NH), 3200 (NH), 1723 (C=O), 1618 (C=N), 1093 (C-O-C); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.05, 11.68 (s, 2H, indole NH), 8.47 (d, 1H, CHCO), 7.08–8.23 (m, 13H, Ar-H), 4.04 (d, 1H, N-CH), 2.13 (s, 3H, CH$_3$); Anal. % $C_{28}H_{20}ClN_5O_2$: C, 68.08; H, 4.08; N, 14.18. Found: C, 68.06; H, 4.03; N, 14.11.

5. Biological Activities

5.1. Antimicrobial Activities. The *in vitro* biological screening of the synthesized compounds (**1** and **3–5**) was carried out against bacterial species, *E. coli*, *B. subtilis*, and *K. pneumonia* and fungal species *A. niger*, *A. flavus*, and *A. fumigates* by cup-plate method [28] using nutrient agar and PDA medium for antibacterial and antifungal activity, respectively. The holes of 6 mm diameter were punched carefully using a sterile cork borer and these were filled with test solution (1 mg/mL in DMF), standard solution (1 mg/mL in DMF), and DMF as control. The plates were incubated at 37°C for 24 hrs and 72 hrs for the evaluation of antibacterial and antifungal activities, respectively. The diameter of the inhibition zones for all the test compounds was measured (in mm) and the results were compared with the results obtained by using streptomycin and fluconazole as positive standard for antibacterial and antifungal activities, respectively.

5.2. Antioxidant Activity Assay

5.2.1. 1,1-Diphenyl-2-picryl Hydrazyl (DPPH) Radical Scavenging Activity (RSA). The radical scavenging activity (RSA) of test compounds (**1** and **3–5**) in methanol at different concentrations (25, 50, 75, 100 μg/mL) containing freshly prepared DPPH in methanol (0.004% w/v) was carried out and the results were compared with the results obtained by using standards (BHA, TBHQ, and AA) by Hatano's method [26]. All analyses were performed in three replicates and results were reported as averaged of three replicates. The results in percentage are expressed as the ratio of absorbance of DPPH solutions measured at 517 nm in the presence and

the absence of test compounds by using ELICO SL171 mini spec spectrometer. The results are shown in Figures 1 and 2. The percentages of DPPH free radical scavenging activity of the samples were determined using the following equation:

$$\% \text{ DPPH radical scavenging} = \frac{A_c - A_s}{A_c} \times 100, \quad (1)$$

where A_c = absorbance of control; A_s = absorbance of test sample.

5.2.2. Ferric Ions (Fe^{3+}) Reducing Antioxidant Power (FRAP). The reducing power of the synthesized compounds (**1** and **3–5**) was determined according to the Oyaizu method [28]. Different concentrations of samples (25, 50, 75, and 100 μg/mL) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1 w/v). The mixture was incubated at 50°C for 20 min. A portion of trichloroacetic acid (2.5 mL, 10% w/v) was then added to the mixture and the mixture was centrifuged for 10 min at 1000 ×g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1 w/v). Absorbance at 700 nm was then measured in spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. The results are shown in Figures 3 and 4.

5.2.3. Ferrous Ions (Fe^{2+}) Metal Chelating Activity. The ferrous ion chelating activities of synthesized compounds (**1** and **3**-**4**) and standards were estimated using the method reported by Dinis and colleagues [29]. The test samples (25, 50, 75, and 100 μg/mL) in ethanol (0.4 mL) were added to ferrous chloride (0.05 mL, 2 mM) prepared in ethanol. The reaction was initiated by the addition of ferrozine (0.2 mL, 5 mM) and the volume was adjusted to 3.5 mL with ethanol and 0.5 mL water so as to make the final total volume 4.0 mL. Ferrozine reacts with the divalent iron to forms stable magenta complex species that were very soluble in water. The mixture was shaken vigorously and kept at room temperature for 10 min. Then the absorbance of the solution was measured spectrophotometrically at 562 nm. All analyses were run in three triplicates and results are reported as the averages of three replicates. The results are shown in Figures 5 and 6. The percent inhibition of the ferrozine-Fe^{2+} complex formation was calculated using the following formula:

$$\% \text{ Ferrous ion chelating effect} = \frac{A_c - A_s}{A_c} \times 100, \quad (2)$$

where A_c = absorbance of control; A_s = absorbance of test sample.

Conflict of Interests

Since the authors have procured the IR, NMR, and mass spectra of the synthesized compounds from the National Research Centre, namely, The Indian Institute of Technology, Madras, Chennai, India, as per the condition of institution authors should acknowledge their services in the research paper while

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